Remarks

Claims 1-33 are pending and claims 7-9 and 31-33 are currently under examination. Claims 1-6 and 1-30 have been canceled as drawn to non-elected inventions. Claims 7 and 31-33 have been amended and new claims 34-38 have been added. Support for claim amendments can be found throughout the specification. In particular, support for "immortalized neural crest cell line" can be found, e.g., at page 12, line 1. Support for "nearly 100%" can be found, e.g., at page 15, line 4. Support for "exogenously added active TGF-β1" can be found at page 3, line 24. No new matter has been added.

Amendment of claims should in no way be construed as an acquiescence to any of the Examiner's rejections. The amendments to the claims are being made solely to expedite prosecution of the present application. Applicants reserve the option to further prosecute the same or similar claims in the instant or in a subsequent patent application.

Applicants respectfully request entry of these claim amendments, since these amendments present the claims in better form for consideration on appeal. The new claims do not raise new issues since the new claims are dependent from claim 7 or 31 and further limit the claimed subject matter.

Rejection of claims 7-9 under 35 U.S.C 101 and 35 U.S.C. 112, first paragraph

The Examiner rejected claims 7-9 under 35 U.S.C. 101 allegedly because the claimed invention is not supported by either a specific or substantial, credible asserted utility or a well established utility, and a person of skill in the art would not know how to use the claimed invention. Applicants respectfully traverse this rejection.

In their previous response, Applicants stated that the claimed invention can be used to identify genes that are up- or down-regulated during differentiation of neural crest cells into smooth muscle cells, which genes can then be used as markers of the stage of differentiation of these cells, and thus, as diagnostic markers, for predicting whether a subject is likely to develop, e.g., an occlusive arteriosclerotic disease. It is the Examiner's position that "[w]hile the method has potential for use as a general research tool which may ultimately result in the development of specific and substantial utilities for identified genes, the discovery process itself does not provide the public with the immediate beneficial knowledge as required to make, practice or perform any specific or substantial product or process within the context of a real world use." The Examiner directed Applicants to the Utility Examination Guidelines, Federal Register, Vol. 66, pages 1092-1099, Friday January 5, 2001.

Applicants respectfully submit that the Utility Examination Guidelines (referred to by the Examiner) do not provide that a "process [that] does not provide the public with the immediate beneficial knowledge as required to make, practice or perform any specific or substantial product or process within the context of a real world use" is not useful. On the contrary, the Revised Interim Utility Guidelines Training Materials, that are posted on the U.S. Patent and Trademark Website, specifically state that "an assay method for identifying compounds that themselves have a 'substantial utility" 'define a 'real world' context of use" (*see*, the definition of "substantial utility"). Thus, since the claimed assay methods identify genes which themselves have a substantial utility, the assay methods define a real world context of use.

Thus, reconsideration and withdrawal of the rejection of claims 7-9 under 35 U.S.C. 101 is respectfully requested.

Rejection of claim 7 under 35 U.S.C. 102(b) as being anticipated by Shah et al.

Claim 7 was rejected under 35 U.S.C. 102(b) as being anticipated by Shah et al. (1996) *Cell* 85: 331. Applicants respectfully traverse this rejection.

The Examiner reiterates the rejection made in the previous Office Action. In the rejection, Shah et al. was relied upon as teaching "mRNA analysis of neural crest cells which are immortalized to the extent that they are capable of multipotent proliferation, and are differentiated to smooth muscle cells." It is the Examiner's position that "the naturally occurring precursors are inherently immortalized as they undergo symmetrical, self-renewing divisions in culture for at least 5-6 days in vitro," and "[t]here is no defining characteristic of an immortalized cell which is absent from the cells of Shah."

Applicants respectfully submit that Shah et al. does not teach immortalized cells. A person of skill in the art would know that a primary culture of cells, which is what the cells of Shah et al. are, are not immortalized, and that they can only be propagated for a limited amount of time in culture. *See, e.g.*, Exhibit A, which are pages 961 and 962 from Molecular Cell Biology, 2nd Ed. by Darnell, Lodish and Baltimore, Scientific American Books (1990), which states that "[w]hen cells are removed from an embryo or adult animal, most of the adherent ones grow continuously in culture for only a limited time before they spontaneously cease growing" (first sentence right column). The Exhibit further indicates that "[v]ery rare cells do not die but continue growing until their progeny overgrow the culture. These cells constitute a cell line, which will grow forever if it is appropriately diluted and fed with nutrients: the cells are immortal" (emphasis added).

Shah et al. discloses rat neural cells that were isolated and cultured as described in Stemple and Anderson (1992) Cell 71:973 (*see,* bottom of left column on page 341, of Shah et

al.), that is, by growing cells from rat embryo trunk sections (see copy of Stemple et al., *supra*, attached hereto as Exhibit B, at page 983, left column "Neural Crest Cell Preparation"). Thus, the cells from Shah et al. are not immortalized, according to the art accepted meaning of this term.

However, merely for expediting prosecution of claims drawn to preferred subject matter, Applicants have amended claim 7 to specify that the neural crest stem cells are an immortalized neural crest stem cell line that is transformed with an oncogene. Shah et al. does not teach or suggest an immortalized neural crest stem cell line that is transformed with an oncogene, and thus, does not teach every element of claim 7.

Thus, reconsideration and withdrawal of the rejection of claim 7 under 35 U.S.C. 102(b) as being anticipated by Shah et al. is respectfully requested.

Rejection of claim 7 under 35 U.S.C. 102(e) as being anticipated by Anderson et al., U.S. Patents 5,672,499 and 6,001,654

Claim 7 is rejected under 35 U.S.C. 102(e) as being anticipated by Anderson et al. U.S. Patents 5,672,499 and 6,001,654. Applicants respectfully traverse these rejections.

The Examiner reiterated the rejection made in the previous Office Action. In the rejection, the Anderson et al. patents are relied upon by the Examiner as teaching "O cells which are smooth muscle cells derived from immortalized neural crest cells and which are recognized to differentially express smooth muscle actin, desmin and calponin in comparison to precursors or neural stem cells differentiated to neurons or glia, as evidenced by antigen expression, see in particular abstract and Example 10." The Examiner also stated that "the cells of the cited references are clonal, proliferative as survive in culture for over 3 days, thus being immortalized."

Applicants respectfully submit that neither of the Anderson et al. patents discloses differentiation of immortalized neural crest cells into smooth muscle cells. As set forth above, primary culture cells (those taught in the two patents) are not capable of proliferating indefinitely in culture, and thus, they are not immortalized cells, as the term "immortalized" is known in the art.

Applicants point out that, contrary to the Examiner's statement, Applicants have not acknowledged that the '654 and '499 references teach immortalized neural stem cells. At most, Applicants had acknowledged that "the references contain a prophetic example indicating how one could immortalize neural crest stem cells," (emphasis added).

The Examiner also stated that "the specification does not teach the extent of uniformly differentiated cells which in comparison to those of the prior art distinguish over the prior art." Applicants respectfully submit that Applicants have obtained populations of smooth muscle cells in which nearly 100% of the cells are smooth muscle cells (see, e.g., page 15, line 4). The claims have been amended accordingly. Neither of the cited patents teaches the uniform differentiation of immortalized neural crest cells into smooth muscle cells. Accordingly, neither of the cited patents teach every element of the claims.

Thus, reconsideration and withdrawal of the rejection of claim 7 under 35 U.S.C. 102(e) as being anticipated by Anderson et al. U.S. Patents 5,672,499 and 6,001,654 is respectfully requested.

Rejection of claims 7-9 under 35 U.S.C. 103(a)

Claims 7-9 have been rejected under 35 U.S.C. 103(a) as being unpatentable over Shah et al. (*Cell* 85:331 (1996)); Anderson et al. U.S. Patent 5,672,499; Anderson et al. U.S. Patent 6,001,654, Baetscher et al. U.S. Patent 5,922,601 and Liang et al. U.S. Patent 5,599,672. Applicants respectfully traverse this rejection.

The Examiner reiterates the rejection made in the previous Office Action. In the rejection, it was the Examiner's position that "[i]t would have been *prima facie* obvious to the skilled artisan as motivated by Baetscher and Liang to study the up and down-regulation of genes in *in vitro* culture systems which have been shown to possess genetic loci which are differentially expressed in the differentiated smooth muscle cells of Shah or Anderson et al. as set forth above utilizing the modified technology of either Baetscher et al. or Liang et al." In the last Office Action, the Examiner stated that "[t]here is no element claimed which is different from the cited references. The cells are immortalized as they are capable of clonal expansion and the cells differentiate uniformly into smooth muscle cells."

As set forth above, Applicants respectfully submit that neither Shah et al., nor either of the Anderson et al. patents teach or suggest a immortalized neural crest cells, as the term "immortalized" is known in the art, which cells differentiate uniformly into smooth muscle cells. Since neither Baetscher nor Liang cure this defect, the cited references fail to teach or suggest all claim limitations.

Applicants further note that it was unexpected that neural crest cells which are immortalized, such as by transformation with v-myc, are capable of differentiating uniformly into smooth muscle cells. In fact, it is known in the art that when primary culture cells are immortalized, the cells frequently loose their potential to differentiate, at least in part since transformation stimulates their proliferation. Accordingly, a person of skill in the art at the time

the application as filed would not have had a reasonable expectation of success in differentiating immortalized neural crest cells. In addition, since it was unexpected that immortalized neural crest cells would still be able to differentiate, there would not have been sufficient motivation to combine the cited references to obtain the claimed invention.

Thus, reconsideration and withdrawal of the rejection of claims 7-9 under 103(a) is respectfully requested.

Rejection of claims 7-9 and 31-33 under 35 U.S.C. 112, second paragraph

Claims 7-9 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The Examiner indicated that "[t]he cells of the prior record are capable in particular of clonal expansion in culture for 3-5 days and are thus considered to be [thus] immortalized" and that "Applicants have failed to provide a limiting definition of that which constitutes an immortalized cell and thus the term appears to be indefinite in the art and specification as Applicants appear to imply for example that an immortalized cell is required to be one which is transformed with an oncogene." Applicants respectfully reiterate the statements made above (and draw the Examiner's attention to Exhibit A), according to which the term "immortal" when referred to cells, is well known in the art to refer to cells which are capable of proliferating indefinitively *in vitro*. One method of immortalizing primary culture cells is by transducing them with an immortalizing gene, e.g., an oncogene (see page 12, first paragraph). Accordingly, the term "immortalized" is definite in the art, and Applicants' use thereof is in conformity with its accepted meaning in the art. Thus, reconsideration of this rejection is respectfully requested.

The claims are also rejected as being incomplete for omitting essential elements, which are "the culture conditions which are sufficient for the neural crest cells to differentiate uniformly into smooth muscle." Applicants respectfully traverse this rejection, and submit that "breadth of a claim is not to be equated with indefiniteness." In re Miller, 441, F.2d 689, 169 USPQ 597 (CCPA 1971). "If the scope of the subject matter embraced by the claims is clear, and if applicants have not otherwise indicated that they intend the invention to be of a scope different from that defined in the claims, then the claims comply with 35 U.S.C. 112, second paragraph." M.P.E.P. 2173.04. The claims are drawn to a method for identifying genes with are up- or downregulated during differentiation of neural crest cells into smooth muscle cells, comprising culturing immortalized neural crest cells under conditions sufficient for the neural crest cells to differentiate into smooth muscle cells, and identifying genes which are up- or down-regulated. Exemplary culture conditions are provided in the specification (e.g., page 12, ines 29-32 and Table 1), and a person of skill in the art could derive similar culture conditions

for differentiating neural crest cells into smooth muscle cells. Thus, the scope of these claims is clear, and the public would be informed of the boundaries of what constitutes infringement of these claims. Reconsideration of this rejection is respectfully requested.

The Examiner also stated that the term "culture conditions" "sufficient" and "differentiate uniformly" in claims 7-9 and 31-33 are relative terms which render the claims indefinite. Applicants respectfully submit that the claims, read in light of the specification, reasonably apprise those skilled in the art both of the utilization and scope of the invention, that the language is as precise as the subject matter permits, and thus that the claims meet 112, second paragraph, requirements. The phrase "differentiate uniformly" is intended to represent that the population of cells resulting from the differentiation consists essentially of smooth muscle cells, and no other types of cells. As indicated in the specification, "differentiate uniformly" includes 100% or nearly 100% differentiation (page 11, lines 27-28).

Regarding the "culture conditions," Applicants submit that these terms are not indefinite, as they refer to medium used for the differentiation of cells. As indicated above, the specification provides exemplary conditions, and persons of skill in the art could derive similar culture media for differentiating immortalized neural crest cell lines.

Accordingly, reconsideration and withdrawal of the rejection of claims 7-9 under 35 U.S.C. 112, second paragraph, is respectfully requested.

Rejection of claims 7, 31 and 33 under 35 U.S.C. 102(b) as being anticipated by Darland et al.

Claims 7, 31 and 33 have been rejected under 35 U.S.C. 102(b) as being anticipated by Darland et al. (May 25, 1996) Dev. Biol. 176: 62. Applicants respectfully traverse this rejection.

The Examiner relies on Darland et al. as teaching "immortalized neural crest cells [obtained] via transformation with the v-myc oncogene," and that "[t]he cells of Darland are not referred to as Monc-1 cells, however the cells are referred to as v-myc transformed neural crest stem cells and are thus deemed to be an equivalent, or inherently the same as Monc-1 cells."

Applicants respectfully submit that Darland et al. does not teach "v-myc transformed neural crest stem cells." Rather, Darland et al. teach "an immortalized cell line from Hensen's node" and that these cells, referred to as "HN-1 cells" "show morphological and immunocytochemical similarities to the prospective mesodermal cells of Hensen's node" (*see*, page 64, right column, second sentence of last paragraph, and sentence bridging pages 71 and 72). Darland et al. teach that conditioned medium from the HN-1 cells are used to study the differentiation of trunk neural crest cells. Thus, since Darland et al. does not teach immortalized neural crest cells, Darland et al. fail to teach every element of claims 7, 31 and 33.

Reconsideration and withdrawal of the rejection of claims 7, 31 and 33 under 35 U.S.C. 102(b) as being anticipated by Darland et al. is respectfully requested.

Rejection of claims 7-9 and 31-33 under 35 U.S.C. 103 as being unpatentable over Darland et al., Shah et al., Anderson et al., Liang et al., Baetscher et al., further in view of Fauquet et al.

Claims 7-9 and 31-33 have been rejected under 35 U.S.C. 103 as being unpatentable over Darland et al., Shah et al., Anderson et al., Liang et al., Baetscher et al., further in view of Fauquet et al., PNAS 87:1546 (1990). Applicants respectfully traverse this rejection.

The Examiner relies on Darland et al., Shah et al., and Anderson et al. as teaching "identification of up- or down-regulated genes in neural crest stem cells induced to differentiate to smooth muscle cells via culture."

The Examiner relies on Liang et al. and Baetscher et al. as teaching "the analysis and cloning of genes in differentiating cells via analysis of mRNA via differential display."

The Examiner further states that "the references do not teach such analysis in neural crest cells which include c-myc oncogene," but states that "Fauquet et al. teach that c-myc, like v-myc transformed cells [...] are immortalized." The Examiner concludes that "in view of the teachings and knowledge of the skilled artisan it would have been prima facie obvious to the skilled artisan to analyze gene expression in c-myc transformed cells undergoing differentiation."

Applicants respectfully submit that Fauquet et al. does not teach that cells transfected with v-myc are immortalized. In addition, Fauquet et al. does not teach that v-myc transformed cells are capable of differentiating, in particular, into smooth muscle cells. As stated above, it is well known in the art that immortalization of primary culture cells is usually accompanied by a loss of the ability of such cells to differentiate. It was unexpected that neural crest cells which are immortalized, such as by transformation with v-myc, are capable of differentiating uniformly into smooth muscle cells. Accordingly, a person of skill in the art at the time the application as filed would not have had a reasonable expectation of success in differentiating immortalized neural crest cells. In addition, since it was unexpected that immortalized neural crest cells would still be able to differentiate, there would not have been sufficient motivation to combine the cited references to obtain the claimed invention.

Thus, reconsideration and withdrawal of the rejection of claims 7-9 and 31-33 under 35 U.S.C. 103 as being unpatentable over Darland et al., Shah et al., Anderson et al., Liang et al., Baetscher et al., further in view of Fauquet et al., is respectfully requested.

Conclusion

In view of the above remarks and the amendments to the claims, it is believed that this application is in condition for allowance. If a telephone conversation with Applicant's Attorney would expedite prosecution of the above-identified application, the Examiner is urged to call the undersigned at (617) 832-1000.

Respectfully submitted,

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Dated: November 9, 2001

Copy of amended claims with changes marked thereon

7. **(Thrice Amended)** A method for identifying a gene which is up- or down-regulated during the differentiation of neural crest cells into smooth muscle cells, comprising

(i) culturing cells of an immortalized neural crest [cells] cell line under culture conditions sufficient for the neural crest cells to differentiate uniformly into smooth muscle cells that are characterized by the expression of smooth muscle α-actin, calponin and SM22α, wherein the neural crest cell line is transformed with an oncogene; and

(ii) identifying genes which are up- or down-regulated under the culture conditions.

31. **(Amended)** The method of claim 7, wherein the [immortalized neural crest cells include an oncogene] culture conditions include the medium identified in Table 1.

32. **(Amended)** The method of claim [31] 7, wherein the [immortalized neural crest cells include c-myc] oncogene is v-myc .

33. **(Amended)** The method of claim [32] 7, wherein the immortalized neural crest cells are Monc-1 cells.

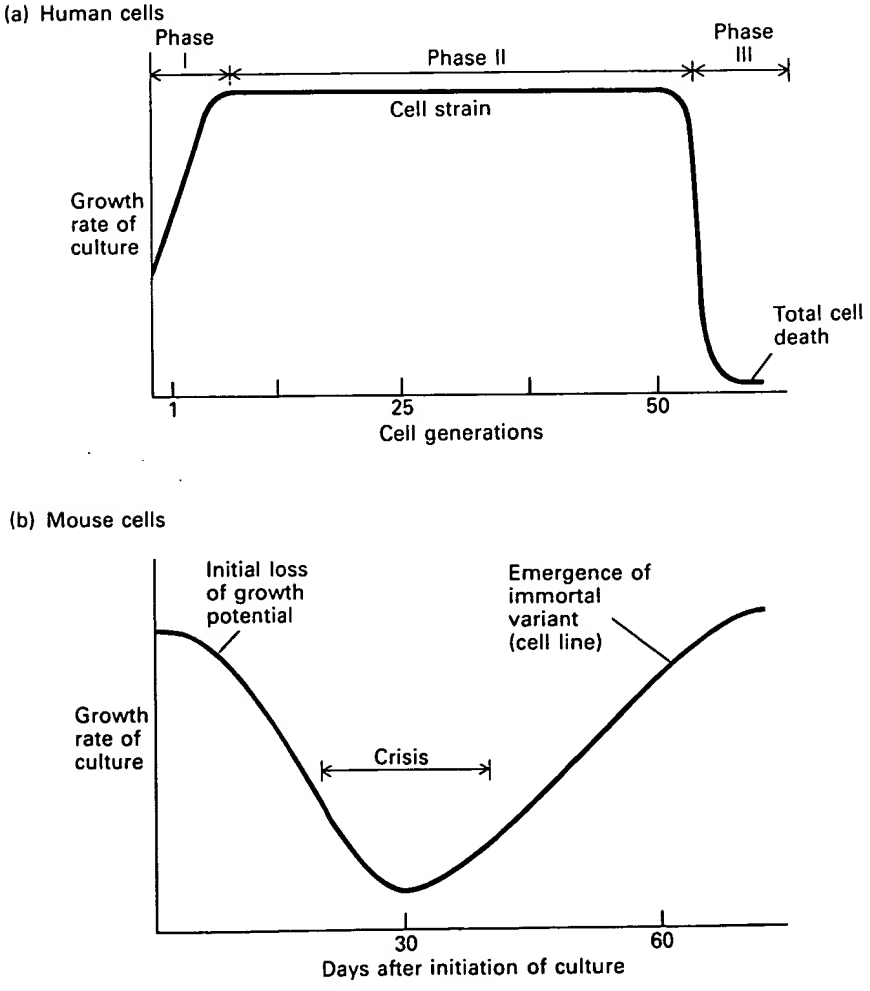
correspond to normal tissue cells, they are representative of the type of cell that comes from the ectodermal or endodermal embryonic cell layers.

Cultured fibrobalsts and epithelial cells are grown on glass or plastic dishes to which they adhere tightly due to their secretion of matrix proteins such as laminin, fibronectin, and collagen. Neither cell type will ordinarily grow if it is not adhering to a substratum. To prepare tissue cells for culture or to remove adherent cells from a culture dish for biochemical studies, trypsin or another protease is used. The process of putting cells into culture or of transferring cells to a new culture is often called *plating*.

Certain cells cultured from blood, spleen, or bone marrow adhere poorly, if at all, to a culture dish. In the body, such nonadherent cells are held in suspension (in the blood) or they are loosely adherent (in the marrow and spleen). Because these cells often come from immature stages in the blood cell lineages, they are very useful for studying the development of leukemias.

Some Cell Cultures Give Rise to Immortal Cell Lines

When cells are removed from an embryo or an adult animal, most of the adherent ones grow continuously in culture for only a limited time before they spontaneously cease growing. Such a culture dies out even if it is provided with fresh supplies of all of the known nutrients that cells need to grow, including blood serum. For example, explanted human fetal cells take some time to become established in culture, during which period the majority of cells die and the "fibroblasts" become the predominant cell type. The fibroblasts then double about 50 times before they cease growth. Starting with 10^6 cells, 50 doublings can produce $10^6 \times 2^{50}$ or more than 10^{20} cells, which is equivalent to the weight of about 10^5 people. Thus, even though its lifetime is limited, a single culture, if carefully maintained, can be studied for a long time. Such a lineage of cells originating from one initial culture is called a *cell strain* (Figure 24-5a).



◀ **Figure 24-5** Stages in the establishment of a cell culture. (a) Human cells. When an initial explant is made (e.g., from foreskin), some cells die and others (mainly fibroblasts) start to grow; overall there is a slow increase in growth rate *(phase I)*. If the remaining cells are continually diluted, they grow as a cell strain at a constant rate for about 50 cell generations *(phase II)*, after which growth begins to slow. The ensuing period of increasing cell death *(phase III)* ultimately leads to the complete death of all of the cells in the culture. (b) Mouse or other rodent cells. When a culture is prepared from mouse embryo cells, there is initial cell death coupled with the emergence of healthy growing cells. As these are diluted and allowed to continue growth, they soon begin to lose growth potential and most cells die (the culture goes into crisis). Very rare cells do not die but continue growing until their progeny overgrow the culture. These cells constitute a cell line, which will grow forever if it is appropriately diluted and fed with nutrients: the cells are immortal.

To be able to clone individual cells, modify cell behavior, or select mutants, it is often necessary to maintain cell cultures for many more than 100 doublings. This is possible with cells from some animal species because these cells undergo a change that endows them with the ability to grow indefinitely. A culture of cells with an indefinite life span is considered immortal; such a culture is called a *cell line* to distinguish it from an impermanent *cell strain*. The ability of cultured cells to grow indefinitely varies depending on the animal species from which the cells originate. For human cells, only tumor cells grow indefinitely, and therefore the HeLa tumor cell has been invaluable for research on human cells. Chicken cells die out after only a few doublings, and even tumor cells from chickens almost never become immortal. With rodent cells, however, cultures of embryonic adherent cells routinely give rise to cell lines.

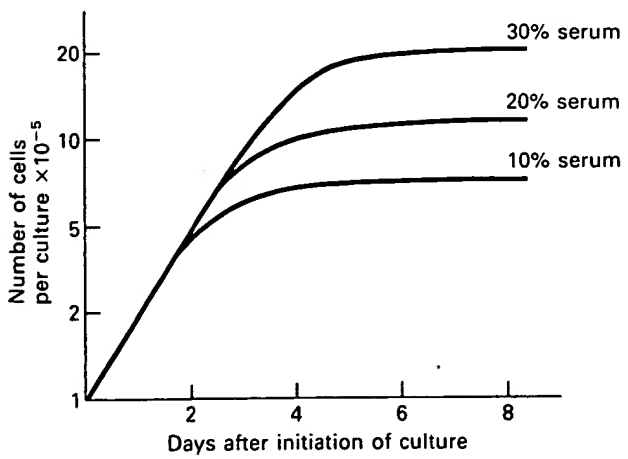
When adherent rodent cells are first explanted, they grow well, but after a number of serial replatings they lose growth potential and the culture goes into *crisis*. During crisis most of the cells die, but often a rapidly growing cell variant arises spontaneously and takes over the culture. Such a variant will grow forever if it is provided with the necessary nutrients (Figure 24-5b). Cells in an established line usually have more chromosomes than the normal cell from which they arose, and their chromosome complement undergoes continual expansion and contraction in culture. The culture is said to be *aneuploid* (i.e., having an inappropriate number of chromosomes), and the cells of such a culture are obviously mutants.

If rodent cell cultures are maintained at a low cell density until a cell line emerges, the line will consist of flat cells that adhere tightly to the dish in which they are grown. A number of mouse cell lines derived in this fashion have been used extensively in cancer research. These lines are called 3T3 cells; they were derived according to a schedule whereby 3×10^5 cells were transferred every 3 days into petri dishes with a 50-mm diameter to maintain the appropriate cell density. As is true for other cultured fibroblasts, the exact cell type that gives rise to 3T3 cells is uncertain, but they can differentiate into a range of mesodermally derived cell types, especially endothelial cells (those that line blood vessels). The ability to derive lines of flat cells like 3T3 set the stage for studying the transition to malignancy in cell culture because cancer cells differ dramatically from 3T3 cells in their growth properties. Before we describe the use of 3T3 cells in cancer research, however, we shall consider the control of 3T3 growth.

Certain Factors in Serum Are Required for Long-term Growth of Cultured Cells

If a culture of 3T3 cells is plated at 3×10^5 cells per dish in a medium with 10 percent blood serum, the cells will grow for a few days and then cease growth at about 10^6 cells per dish. The culture is said to have reached *saturation density*. Although the quiescent cells in a saturated culture have stopped growing, they can remain viable for a long time and resume growth if supplied with fresh medium.

Among the treatments that will reinitiate growth in a quiescent 3T3 cell culture is the addition of extra serum to the medium. In fact, the density at which the cells stop growing is in direct proportion to the amount of serum with which they are initially provided (Figure 24-6). Although this result appears to indicate that serum factors are the primary determinants of whether cells remain quiescent or initiate growth, other results show that they are not the whole story. For example, if a strip of cells is removed from a quiescent cell culture (the culture is said to be *wounded*), the cells at the border of the wound will begin growing and will divide a few times to fill the gap. Because the cell medium is not altered in such an experiment and because the cells that are not adjacent to the wound do not initiate growth, it is clear that local effects (cell-to-cell contacts) also control cell growth. In all prob-



◀ **Figure 24-6** The dependence of cell growth on serum concentration. A constant number of 3T3 cells was used to initiate multiple cultures, each of which was fed with a medium containing the indicated percentages of fetal calf serum. The number of cells per culture was determined daily. The initial growth rates were indistinguishable, but the final number of cells was proportional to the amount of added serum. The experiment shows that serum factors rather than cell contacts control cell growth because cells are already touching one another in 10% serum. [See R. W. Holley and J. A. Kiernan, 1968. *Proc. Nat'l Acad. Sci. USA* 60:300.]